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Fatty Acid Synthetase of *Saccharomyces cerevisiae*

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A light particle fraction of *Saccharomyces cerevisiae*, obtained from the crude ribosomal material, and containing the fatty acid synthetase, consisted primarily of 27*S* and 47*S* components. This fraction has a protein-ribonucleic acid ratio of about 13. Electron micrographs showed particles ranging in diameter between 100 and 300 A in this material. By use of density gradient analysis, the fatty acid synthetase was found in the 47*S* component. This component contained particles which were predominantly 300 A in diameter and which were considerably flatter than ribosomes, and it consisted almost entirely of protein.

In a recent report (7), it was shown that the crude ribosomal fraction, sedimented at 100,000 $\times$ *g* from a mitochondria-free homogenate of *Saccharomyces cerevisiae*, was capable of incorporating acetate into fatty acids but not into nonsaponifiable lipids. It was also found that the crude ribosomal particles were separable into a "light" fraction, containing fatty acid synthetase activity, and a "heavy" fraction, containing acetokinase (acetyl-coenzyme A synthetase) and fatty acid desaturase activity. In addition, the "heavy" fraction is known to be rich in the squalene oxidocyclase system (H. P. Klein and C. M. Volkmann, *unpublished data*).

Centrifugation studies (7) with this strain of *S. cerevisiae* suggested that the fatty acid synthetase sedimented as a 45*S* particle, although no direct determinations were made on the size of this enzyme complex. Lynen and his coworkers (8-10) purified a particle from commercial bakers' yeast by ammonium sulfate fractionation, adsorption on and elution from calcium phosphate gel, further ammonium sulfate fractionation, and treatment with alumina gel. The resultant purified fatty acid synthetase was estimated to be a 43*S* particle with a molecular weight of about 2.3×10^6.

In the present investigation, the "light" particle fraction of *S. cerevisiae*, containing the fatty acid synthetase, was further fractionated by centrifugation procedures. Two particle populations were found to constitute the bulk of this fraction. Of these, only one has fatty acid synthetase activity.

MATERIALS AND METHODS

S. cerevisiae strain LK2G12 was the organism used in these studies. Details concerning its cultivation, the preparation of cell-free homogenates, and methods for isolating the fatty acids are given in earlier publications (6, 7). Fatty acid synthetase activity was measured by the incorporation of malonyl coenzyme-A-*1,3*-^{14}C (malonyl-CoA) into fatty acids (10).

"Light" particles were obtained (7) by suspending the crude ribosomal particles, obtained from mitochondria-free homogenates, over 10% sucrose solution and centrifuging at 30,000 rev/min for 65 min with a no. 30 Spinco rotor. Under these conditions, the "heavy" particles (containing ribosomes and membranes) sedimented at the bottom, and the "light" particles remained suspended above the sucrose and could be obtained subsequently by centrifuging the top layer. A simpler method of obtaining "light" particles was to wash the crude ribosomal particles twice in a buffer composed of 0.002 M tris(hydroxymethyl)aminomethane (*p*H 7.6) plus 0.002 M $MgCl_2$, sedimenting the particles at 100,000 $\times$ *g* for 60 min each time. The combined washings, when centrifuged at 100,000 $\times$ *g* for 90 min, yielded somewhat more "light" particle material than the method previously described, but the yield was sometimes contaminated with small amounts of ribosomal material, as evi-

TABLE 1. *Protein and RNA content of "light" fractions*

Sample	Protein	RNA	Protein-RNA ratio
	mg/ml	*mg/ml*	
S-3	1.5	0.10	15.0
S-4	2.1	0.16	13.1
S-5	11.0	1.10	10.0
S-6	8.4	0.48	17.5
S-7	11.0	0.97	11.4
E-9[a]	2.9	0.28	10.4

[a] Obtained by layering on 10% sucrose. Other preparations were obtained by the washing technique.

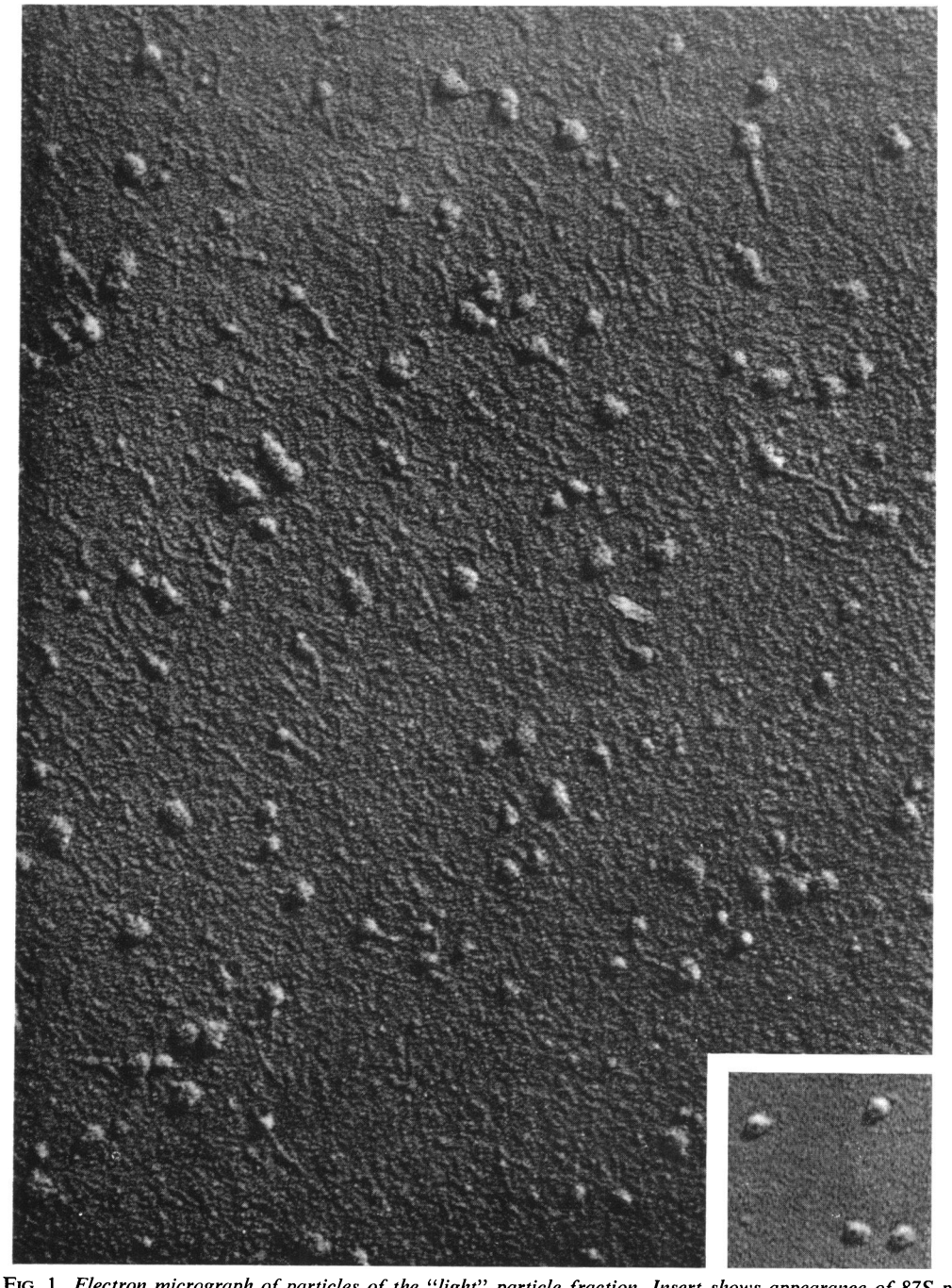

FIG. 1. *Electron micrograph of particles of the "light" particle fraction. Insert shows appearance of 87S particles of "heavy" fraction for comparison. Both photographs were taken at the same magnification (× 100,000) and were shadowed at the same angle.*

denced by the appearance of an 87*S* component upon analytical ultracentrifugation.

A sucrose gradient (10 to 50%) was used in the density gradient analyses of the "light" particle preparations. After the preparations were centrifuged at 124,000 × *g* for 16 hr, the resultant tubes were scanned with a recording ultraviolet analyzer (model UA; Instrumentation Specialties Co.) at 254 mμ,

and fractions were collected in a density gradient fraction collector (model D; Instrumentation Specialties Co., Lincoln, Neb.). Proteins and ribonucleic acid (RNA) were determined either by the Lowry and orcinol methods, respectively, or spectrophotometrically; the analytical procedures are referred to in earlier publications (5–7).

Electron microscopy was performed with a Hitachi model H10 electron microscope. For this, particle preparations were diluted with double-distilled water to a final concentration of 0.01 to 0.02 mg of protein per ml, sprayed onto grids, and then shadowed with uranium at an angle of 26°.

For analytical ultracentrifugation studies, the "light" particle preparations were diluted to yield a protein concentration of 1.5 to 5 mg/ml. The Spinco model E ultracentrifuge was used in these analyses.

RESULTS

General properties of "light" particles. In contrast to the "heavy" fraction, which consistently yielded a protein-RNA ratio of about 1.8 (corresponding to about 65% protein and 35% RNA), the "light" particles obtained by both methods described above gave protein-RNA ratios of 10 or higher (Table 1).

Electron microscopy of "light" particle preparations revealed particles with diameters ranging from about 100 to 300 A. Particles with diameters of 250 to 300 A and showing longer shadows (characteristics of the 87*S* particles of the "heavy" fraction) were also observed in this material (Fig. 1).

It was demonstrated (Table 2) by use of the analytical ultracentrifuge that the "light" particle fraction consistently contained two main sedimenting boundaries at approximately 27*S* and 47*S* (usually accompanied by trace amounts of 87*S* particles). The sedimentation coefficients of the 27*S* and 47*S* particles were relatively independent of concentration, a fact strikingly different from the behavior of the 87*S* particles found in the "heavy" fraction. Table 2 also shows that treatment of the "light" particle fraction with ribonuclease did not affect the sedimentation characteristics of the 27*S* and 47*S* particles. Such treatment, however, effectively aggregates 87*S* particles, causing them to precipitate out very readily (H. P. Klein, C. M. Volkmann, and J. Weibel, *unpublished data*). Figure 2 shows the ultracentrifugal patterns of a preparation of "light" particles after ribonuclease treatment. No trace of the 87*S* component remained after this treatment.

Fatty acid synthetase activity in the "light" particle fraction. When preparations of the "light" particles were subjected to density gradient centrifugation, fatty acid synthetase activity was contained in a narrow region of the gradient. Figure 3 shows the results of one such experiment. Two protein peaks were obtained that approximated the 27*S* and 47*S* components seen in the analytical ultracentrifuge. However, the fatty acid synthetase activity was found almost exclusively in the peak corresponding to the 47*S* component. When the pooled material in tubes 11 to 13 was collected and analyzed in the ultracentrifuge, a single component, having a sedimentation coefficient of 47*S*, was obtained. A suspension of these 47*S* particles contained, per ml, 0.36 mg of protein and 0.011 mg of RNA, giving a protein-RNA ratio of about 33, which was considerably higher than that of the starting material (*see* Table 1). Figure 4, a shadowed preparation of this material, shows numerous relatively uniform particles approximately 300 A in diameter. These particles appear quite flat, unlike the 87*S* particles (shown in the insert). The latter, in addition, are spherical. Particles smaller than 200 A in diameter were also present in this preparation, presumably indicating some contamination by 27*S* particles.

DISCUSSION

The data presented here indicate that the fatty acid synthetase of *S. cerevisiae* can readily be

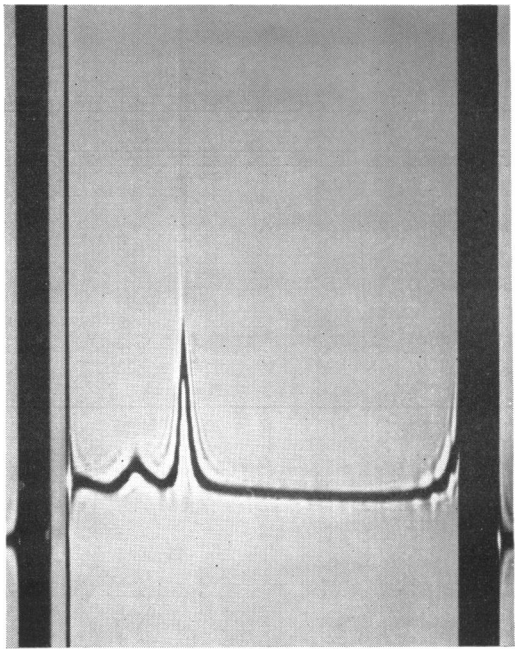

FIG. 2. *Ultracentrifuge pattern of ribonuclease-treated "light" particles showing 27S (left) and 47S (right) boundaries. Pictures were taken 8 min after reaching a speed of 42,040 rev/min; bar angle, 60°; temperature, 22.8 C.*

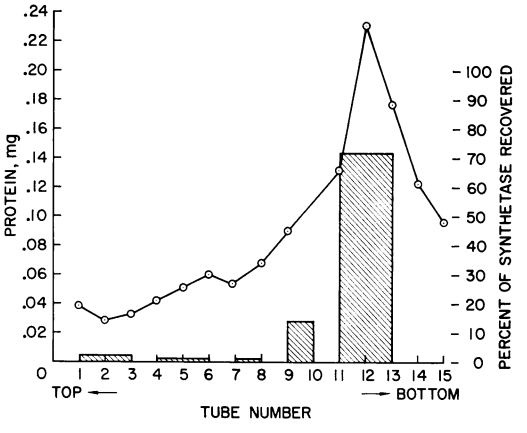

FIG. 3. *Separation of fatty acid synthetase on sucrose density gradient. A 0.1-ml amount of "light" particles, containing 1.4 mg of protein, was layered over the gradient. After centrifugation, 15 fractions were collected. Protein was determined in each fraction, and fatty acid synthetase was assayed in pooled fractions, as shown. Protein recovered in fractions, 95%; synthetase recovered, 94%.*

TABLE 2. *Sedimentation coefficients of sedimenting boundaries of "light" particle preparations*

Sample	Protein concn	Sedimentation coefficient ($S_{20,w}$)
	mg/ml	
F-36	3.0	27 46 85 (trace)[a]
	1.5	27 45 84 (trace)[a]
F-33	3.0	28 45 86 (trace)[a]
F-33[b]	3.0	27 46
F-41	5.0	26 45 84 (trace)[a]
F-41[b]	5.0	27 47
F-49[b]	3.0	27 47
	1.5	27 47

[a] Detailed studies on this component (Chao, *unpublished data*) indicate that the actual sedimentation coefficient is somewhat higher (87*S*) than observed in the analyses shown here.

[b] These samples were first treated with 0.1 mg of ribonuclease per ml at 25 C for 2 hr. A slight cloudiness which developed was centrifuged off prior to ultracentrifuge analysis.

obtained from homogenates by centrifugation, and that this enzyme sediments as a particle with a sedimentation coefficient of approximately 47*S*. The data thus support the findings of the Lynen group and, furthermore, indicate that the fatty acid "complex" as described by Lynen (8–10) exists in situ and is not simply an artifact of protein purification. We cannot, of course, conclude that all of the 47*S* component observed here is composed of the fatty acid synthetase.

Of general interest may be the fact that the "light" particle fraction of these homogenates appears to contain a variety of particles. Although this fraction may well contain ribosomal subunits, the relatively low RNA content, the insensitivity to ribonuclease, and the lack of a concentration effect on sedimentation behavior are not characteristic of the ribosomal particles. The bulk of particles in the "light" fraction, therefore, seem to have no direct relation to the ribosomes. Approximately 2 to 7% of the total protein of the crude particulate material obtained from postmitochondrial homogenates is in this "light" fraction, and, in view of the large quantity of ribosomes and membranes (Klein et al., *unpublished data*) in the crude particles, the particles of the "light" fraction represent a significant portion of the cellular apparatus.

Very little can be said at this time about what enzymatic or other functions are carried out by the particles of the 27*S* component of the "light" particle fraction. Preliminary density gradient centrifugation studies (Klein and Rasmussen, *unpublished data*) indicate that acetyl-coenzyme A carboxylase is present in this component. It is also of interest that, when clean preparations of ribosomes (87*S* particles) are exposed to high phosphate concentrations that dissociate these particles (2), no 27*S* component is observed among the fragments of the original particles (Chao, *unpublished data*).

In an earlier report from this laboratory (6), it was assumed that the bulk of the membranes of postmitochondrial homogenates would be found in the "light" particle fraction. This contention was based on the fact that this fraction was found to be relatively rich in total lipids compared with the "heavy" particles (5). More recent work, however, with ^{14}C-choline as a tracer for phospholipid-containing membranes, indicates that the "light" particles are completely free from membranous material, virtually all of the phospholipid being found in the "heavy" particle fraction (Klein and Volkmann, *unpublished data*). The lipids reported to be found in the "light" particle fraction probably represent neutral glycerides and are not representative of membranes. The point to be emphasized here is that the fatty acid synthetase does not appear to be associated with membranes as do some of the other enzymes involved in lipid synthesis in this organism (Klein and Volkmann, *unpublished data*). In this connection, it should also be mentioned that we have been unable to demonstrate fatty acid synthetase activity in preparations of the mitochondrial fraction of this yeast. Other investigators (1, 3, 4, 11) have reported that cer-

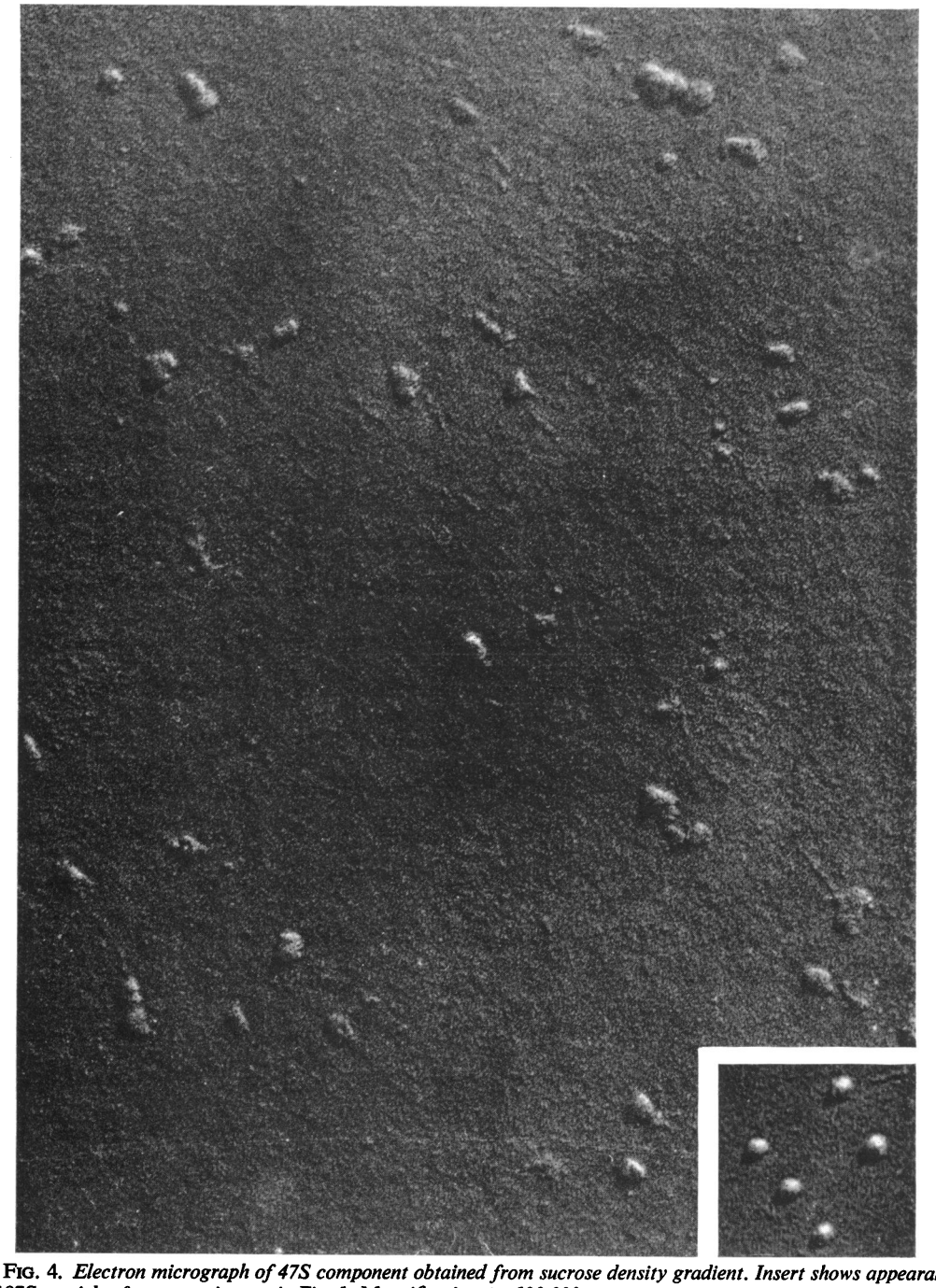

FIG. 4. *Electron micrograph of 47S component obtained from sucrose density gradient. Insert shows appearance of 87S particles for comparison as in Fig. 1. Magnification, × 100,000.*

tain animal and plant mitochondrial preparations are capable of forming fatty acids.

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